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LIFE CYCLE OF LABORATORY-REARED TOBACCO HORNWORMS, *MANDUCA SEXTA*, A STUDY OF DEVELOPMENT AND BEHAVIOR, USING TIME-LAPSE CINEMATOGRAPHY

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The tobacco hornworm, *Manduca sexta* (L.), is a multivoltine leaf feeder that grows rapidly up to 10 g in the larval stage and has been studied since the start of the tobacco industry. The development of controlled laboratory rearing techniques (Yamamoto, 1969; Bell and Joachim, 1976) and its large size have promoted the use of this insect as a general lepidopteran representative for many biological studies. There is now an extensive accumulation of knowledge about *M. sexta* from field studies and from physiology and endocrine studies (Madden and Chamberlin, 1945; Peterson, 1912; Reinecke, Gerst, O'Gara, and Adams, 1978; Truman, 1972; Borg and Marks, 1973; Bell, Rasul, and Joachim, 1975; Bollenbacher, Vedeckis, Gilbert, and O'Connor, 1975; Sandburg, Kramer, Kezdy, and Law, 1975; and Kramer, Dunn, Peterson, Seballos, Sandburg, and Law, 1976). However, a complete description of the developmental and behavioral events of this insect in the laboratory has never been reported. Such information is extremely important in the rearing and use of this insect for research and when more precise staging of physiological age is needed. Accurate staging is critical in endocrine studies of lower animals such as insects because hormone releases are usually of very short duration and frequently induce a rapid change in behavior (Truman and Riddiford, 1977; Truman and Endo, 1974).

This paper reports the application of time-lapse cinematography for observations on growth, development, and behavior of laboratory-reared specimens of *M. sexta* to determine more accurate timing of morphological, physiological, and behavioral events.

Materials and Methods

Rearing procedures

Specimens of *M. sexta* were reared by the method of Bell and Joachim (1976), but the diet used was a high wheat germ type developed for mass rearing the gypsy moth, *Lymantria dispar* (L.) (Bell, Owens, Shapiro and Tardif, 1980). This diet was altered to contain 0.24% formaldehyde and 10% less water (to increase the firmness of the congealed diet). Rearing temperature was maintained at 25° ± 0.2° C, and the relative humidity was held between 20 and 40%. A diapause-inducing light regimen was used (12L:12D). Lights-off was at 6:00 P.M., which was arbitrarily designated as 2400 (00:00) A.Z.T. (Truman, 1972).

Preparation of containers for photography

Standard rearing cups (Bell and Joachim, 1976) were used for multiple photographic recordings of up to 11 insects. The 30-ml cups were placed on their

sides, and the 120-ml cups were placed upside down, which allowed photographic observations at low magnifications.

For more detailed observations at higher magnification, two sizes of containers were constructed from clear plexiglass tubing with 3.2 and 7.0 cm inside diameters. They were 5 and 8 cm high, respectively. Both were covered with glass lids and placed on a plexiglass sheet that served as the container bottoms and the photographic platform. The bottom of the 3.2-cm container was covered with a 5-mm thick layer of diet, which was sufficient to maintain the larvae through the fourth instar. The larger container was used for fourth-instar insects through pupation and blocks of diet were supplied every two days (at which time the frass was removed) until feeding stopped. At the cessation of feeding, the bottoms of the containers were occasionally lined with tough brown absorbent paper.

A third photographic setup was constructed from two 70 × 50-cm glass plates that were separated by 2 cm with a wooden frame. Three-fourths of this container was filled with soil and placed on the long edge for time-lapse photography of nondiapausing fifth-instar insects from cessation of feeding through pupation and again at 18 days to record eclosion.

During filming, an electric wrist watch with date, a small centigrade thermometer, a 20-mm ruler, and a data card were placed in the field of view of the cylindrical containers. A clock was used with the soil-filled container.

Procedures for selection of insects

Except where noted, only the physiologically advanced (PA) specimens of *M. sexta* were used. The PA insects represented about 10% of the total population at the time of this study and were chosen because of their greater synchrony and uniformity as a group and the ease in which they could be selected.

When an entire larval cycle was recorded by time-lapse cinematography, there was a slight retardation. This was avoided by recording only a part of the life cycle at any one time and comparing it with the progress of the insects remaining within the standard colony. Thus, timing records were compiled from a number of filmed sequences with durations of 6 to 12 days.

Eggs for the more critical time studies were collected from a tobacco plant that had been in the egging chamber between 11:30 PM and 12:30 AM. Insects hatching from these eggs were photographed through the second ecdysis only. Records were made for only the most rapid developers.

Photographic equipment

An Arriflex 16-mm movie camera adapted for time-lapse and flash synchronization was used for all filmed studies. The photographic illuminator was a Chadwick-Helmuth lamp (model 71) powered by their Strobex 136–M1. The electronic flash was used without the accompanying condenser lens, and a flash meter (Calumet M-100) was used to measure the light output for film exposure (the film was Eastman 7240, 16-mm ASA 80, reversal color with 85B filter). A filming rate of one exposure per five min was commonly used for the time-lapse recordings. The film was viewed with an L.W International data anlyzer projector (224 AMK IV).

Environmental conditions for photography

Filming of the animals was conducted in a controlled environmental room at the same conditions as described for rearing. Since diapause in *M. sexta* is

inhibited by a light interruption applied shortly after the onset of the dark period (Bell, Rasul, and Joachim, 1975), effects of the electronic flash on diapause was examined by placing the electronic flash 60 cm from a group of 20 newly ecdysed fifth-instar larvae. The flash was triggered every 30 sec at maximum intensity (about 75 joules/flash with a flash duration reported to be 135 microseconds) for the duration of the instar. All insects entered diapause, and no other effect of the flash was noted.

Analytical procedures

The date and time of day were recorded from the films for each event. Time from oviposition (chosen as 12:05 AM on the night oviposited) to each event was determined, and means and standard deviations were calculated for the onset of events and, again, for the duration of events. The data from obviously aberrant insects were discarded. The percentage of time elapsed to pupation and A.Z.T. times were derived from the data on time to occurrence. Data on average weight were obtained from standard colony insects (not just PAs) that were selected during known weight-stable stages.

TABLE I

Time of onset of events in days, percentages, and A.Z.T. time for physiologically advanced (PA)† specimens of* M. sexta.

Event	Time at onset of events in days	N	Percentage of development at onset of events	A.Z.T. time of day in hr
Oviposition	00.00 ± 0.01‡	11	00.00 ± 0.05‡	06.0 ± 0.3‡
First-instar hatch (L1)	4.03 ± 0.02	4	17.71 ± 0.11	06.8 ± 0.6
Molt sleep	5.78 ± 0.02	4	25.38 ± 0.09	00.7 ± 0.5
L1-L2 ecdysis	6.37 ± 0.02	11	27.96 ± 0.08	14.8 ± 0.4
Molt sleep	7.82 ± 0.06	10	34.36 ± 0.27	01.8 ± 1.5
L2-L3 ecdysis	8.41 ± 0.06	10	36.92 ± 0.26	15.8 ± 1.4
Molt sleep	9.97 ± 0.10	8	43.79 ± 0.41	05.3 ± 2.3
L3-L4 ecdysis	10.65 ± 0.10	8	46.75 ± 0.42	21.5 ± 2.3
Molt sleep	12.68 ± 0.09	7	55.69 ± 0.41	22.3 ± 2.2
L4-L5 ecdysis	13.71 ± 0.11	11	60.21 ± 0.50	23.0 ± 2.7
Coated fecal pellets	15.76 ± 0.04	4	69.21 ± 0.18	00.2 ± 1.0
Cessation of feeding	17.61 ± 0.15	11	77.32 ± 0.69	20.5 ± 3.7
Heart exposure	17.72 ± 0.12	5	77.83 ± 0.53	23.4 ± 2.9
Body wetting	17.81 ± 0.08	11	78.20 ± 0.36	01.4 ± 1.9
Wandering	17.92 ± 0.08	11	00.00 ± 0.05	06.0 ± 0.3
Dorsal pigmentation	17.98 ± 0.20	3	78.94 ± 0.88	05.4 ± 4.8
Burrowing	18.27 ± 0.19	11	80.25 ± 0.85	12.6 ± 4.6
Fluid excretion	18.61 ± 0.22	7	81.73 ± 0.98	20.6 ± 5.4
Reduced movement	19.23 ± 0.20	11	82.59 ± 0.88	01.3 ± 4.8
Stationary stage	19.75 ± 0.10	11	86.74 ± 0.42	00.0 ± 2.3
Metathoracic bars	22.11 ± 0.20	11	97.11 ± 0.87	08.7 ± 4.8
Larval-pupal ecdysis	22.77 ± 0.20	11	100.00 ± 0.88	00.5 ± 4.8

* A.Z.T. time is 0 at onset of 12-hr scotophase, which was 6:00 PM in this study. Thus heart exposure occurred 23.4 hr after onset of scotophase and took place in photophase, of which there were 0.6 hr left.

† PA = insects selected as most physiologically advanced (about 8 or 9% faster than standard colony), held at 25° C and 12-hr photophase.

‡ Data represent the mean and s.d. for the numbers of insects (N) as indicated.

TABLE II

Duration of events of PA-selected specimens of* M. sexta *and weight of non-PA selected insects during weight-stable stages.*

Event	Mean duration of events in hr	N	Mean weight	N
Egg stage	96.8 ± 0.6**	8	1.41 ± 0.10 mg**	20
First-instar feeding	42.0 ± 0.7	4	1.03 ± 0.07 mg†	11
Molt sleep	14.1 ± 0.5	4	7.64 ± 1.29 mg	20
Second-instar feeding	35.0 ± 1.9	11		
Molt sleep	14.0 ± 1.6	11	30.40 ± 2.70 mg	8
Third-instar feeding	37.5 ± 3.7	11		
Molt sleep	16.2 ± 0.8	6	0.20 ± 0.02 g	21
Fourth-instar feeding	46.6 ± 0.9	7		
Molt sleep	24.7 ± 2.0	8	1.08 ± 0.09 g	20
Fifth-instar feeding	90.6 ± 3.8	11		
Cessation of feeding to body wetting	4.8 ± 1.6	11	9.23 ± 0.53 g	10
Body wetting	2.7 ± 0.7	11		
Wandering to burrowing	8.5 ± 2.9	11		
Dorsal pigmentation††	27.0 ± 8.4	3		
Fluid excretion††	28.3 ± 7.4	6		
Burrowing to reduced movement	22.9 ± 5.5	11		
Reduced movement	12.9 ± 3.9	9		
Stationary stage to pupation	72.5 ± 4.3	11		
Metathoracic bars to pupation††	15.8 ± 1.4	11	5.02 ± 0.63 g	10
Pupation to eclosion—diapause	2,328 ± 720‡	567	4.71 ± 0.62 g	30
Pupation to eclosion—nondiapause	523 ± 31‡	26		

* Insects selected as most physiologically advanced (~10% of colony population), held at 25° C and 12-hr photophase.

** Data represent the mean and s.d. for the number of insects (N) as indicated.

† Weight taken immediately after hatch.

†† Overlapping events.

‡ Duration of diapause (obtained from one-month data of standard colony) = 97 ± 30 days. Duration of nondiapause (induced with 15 hr light: 9 hr dark) = 21.8 ± 1.3 days.

RESULTS

Data obtained from filming the growth and development of individual laboratory-reared specimens of *M. sexta* were summarized, and the timing for major physiological and behavioral events is presented in tabular form. Table I lists the events monitored in order of occurrence, the mean number of days per event, and the mean percentage of time elapsed btween events from oviposition to pupation (100%). Table II shows the mean duration of most events in hours in order of occurrence, with the mean weights of insects selected from the standard colony during known weight-stable stages.

Oviposition

A time-lapse recording was made of adult insects in the egging cage in an effort to determine whether there were any general activity cycles. Flying adults were momentarily affected by each electron flash (5-min intervals), but egg harvest was normal. It was found that oviposition, periods of rest, feeding, and mating occurred randomly under simulated moonlight conditions (dimmed tungsten bulb emitting 3 lux at 20 cm) throughout the 12-hr dark period. Activities started and stopped about five min after the onset of the dark and light cycles, respectively, and there was no effective activity during the light period. Thus, oviposition

occurred between 6:05 PM and 6:05 AM, and the average time of oviposition for all specimens was assumed to be 12:05 AM.

Larval growth and development

Egg hatch and larval ecdyses. Larval emergence from the egg occurred 4 days after oviposition (Table I), and the mean weight immediately after hatch was about 1.4 mg (Table II). The first-instar larvae had silk glands that produced a strand on which they could descend. Also, a small mass of silk was frequently found tangled around the prolegs at the end of this instar. Ecdysis took less than 1 min to complete for all larval stages, as observed by eye. Between the onset times of larval ecdyses (Table I), the average length of time spent in the first through fourth instars was 2.3, 2.0, 2.2, and 3.1 days, respectively.

Feeding. Immediately after egg hatch, most larvae consumed their egg cases before migrating a short distance to feed on diet. During the first instar, larvae fed for a few minutes, withdrew their heads from the food, and then either remained motionless or moved the head and thorax regions back and forth. The first-instar feeding stage lasted about 42 hr from eclosion, and during this time, the insects showed a 7.4-fold increase in weight (Table II). Second, third, and fourth instar larvae frequently ingested part of their cast skin soon after ecdysis and before feeding on diet. The feeding stages from eclosion for these three instars lasted 35, 38, and 47 hr, respectively (Table II). Film records of newly eclosed fourth and fifth instars showed a feeding delay of 2.3 ± 1.0 hr ($n = 8$) and 2.9 ± 2.0 hr ($n = 10$), respectively. Similar delays in feeding were assumed for the first through third instars. Feeding behavior during the fifth instar was studied in detail; a single fifth-instar larva was studied over a 45-hr period, and durations of feeding and nonfeeding were highly variable. For this larva, mean duration of a feeding period was 15 ± 20 min ($n = 71$ consecutive feedings), and duration of a nonfeeding period was 20 ± 20 min ($n = 70$). The majority of feeding contacts was of short duration, lasting about 5 min, and the longest was 150 min. Another fifth-instar larva fed at mean intervals of 41 ± 56 min ($n = 21$ consecutive feedings), and had mean nonfeeding intervals of 19 ± 13 min ($n = 21$ intervals) over a 24-hr period.

The feeding stage of all instars was easily interrupted by external disturbances such as the approach of an observer. Such interruptions were most noticeable for the larger fifth-instar larvae. When startled, the larvae remained motionless for several minutes; thus, frequent disturbances could possibly affect growth rate. To determine whether the photographic procedure was seriously affecting feeding behavior, fourth- and fifth-instar larvae, (shortly after ecdysis) were allowed to feed under standard rearing conditions for 40 hr. Then the number of fecal pellets was counted. In all instances, the number of pellets was similar to those determined by film analysis. Thus, the photographic apparatus did not interfere with feeding. The highest rate of pellet production observed by film analysis was one every 19 ± 3 min ($n = 41$) for fifth-instar larvae the third day after ecdysis Pellet production was not critically determined for the first three instars.

Rate of growth and weight-doubling time. Fifth-instar larvae gained 90% of their weight (9.23 g) during a feeding period of 91 hr (Table II). However, this instar had the slowest rate of growth, as shown by a weight-doubling time of 21.2 hr (Table III). A weight-doubling time of 17.6 hr for second-instar larvae was more than 1.5 times as long as that determined for first- and third-instar larvae although it was the same as for the fourth instar. Figure 1 depicts a graphic

TABLE III

Feeding time per instar needed for M. sexta *larva to double in weight.*

Instar	Weight-doubling time (hr)*
First	11.3
Second	17.6
Third	11.4
Fourth	17.3
Fifth	21.2

* Duration of each feeding stage derived from Table II as well as weight at hatch, during molt sleep, and at cessation of feeding of fifth-instar larvae.

perspective between growth rate and life cycle activities through pupation. The rapid growth rate for first and third instar larvae is easily seen by the slope of the plot of log of percentage weight vs. percentage of time (solid line, Fig. 1). The massive weight gain of fifth-instar larvae is clearly illustrated when viewed as a linear scale and compared with the other instars (dashed line, Fig. 1).

Molt sleep. At 5.8 days after oviposition, the first-instar larvae entered a quiescent period—the molt sleep. The onset of this event occurred within a period of 10 min and was followed by a slight body pulsation that lasted about 15 min. The pulsation was associated with the displacement of the old head capsule. Similar molt sleep behavior was observed for all but fifth-instar larvae. Duration of this event was 14 to 16 hr for the first through third instars and increased to about 25 hr during the fourth instar (Table II).

Truman (1972) observed gating in the initiation of the molt to the next instar, where about half the second-instar insects initiated the molt to the third instar during the first night after the second-instar ecdysis, but the remaining insects did not until the second night. In the present study, gating was not observed in the PA insects, but the phenomenon was routinely observed in the standard colony.

Coated fecal pellets. Midway through the feeding stage, fifth-instar larvae produced fecal pellets that were coated with a chalky material identified as uric acid (Buckner, Caldwell, and Reinecke, 1980). Nijhout and Williams (1974) were the first to report this phenomenon, which they termed "frosted frass," and they observed it after 3 days of feeding into the fifth instar. The high wheat germ diet used in this study did not produce markedly coated pellets. To increase the contrast of the coating, 2 mg/ml of powdered charcoal was added to some fresh diet. The larvae were transferred to the resulting black diet 1 day after the fourth to fifth ecdysis. The insects were filmed from ecdysis to beyond the feeding stage. The film showed two periods of coating: the first was compartively light and occurred at 2.08 ± 0.04 ($n = 4$) days after ecdysis. The second and heavier coating occurred at 2.44 ± 0.10 ($n = 4$) days post ecdysis. The onset of these two events was synchronous within the group photographed, and the first coated frass coincided with the onset of scotophase. Coated fecal pellets were produced throughout the remainder of the feeding period, but there was variability of coating between pellets that ranged from heavily coated to uncoated.

Development from post feeding through pupation

Cessation of feeding. Near the end of the fifth-instar feeding stage, the rate of defecation decreased. Feeding activity abruptly stopped about 94 hr after

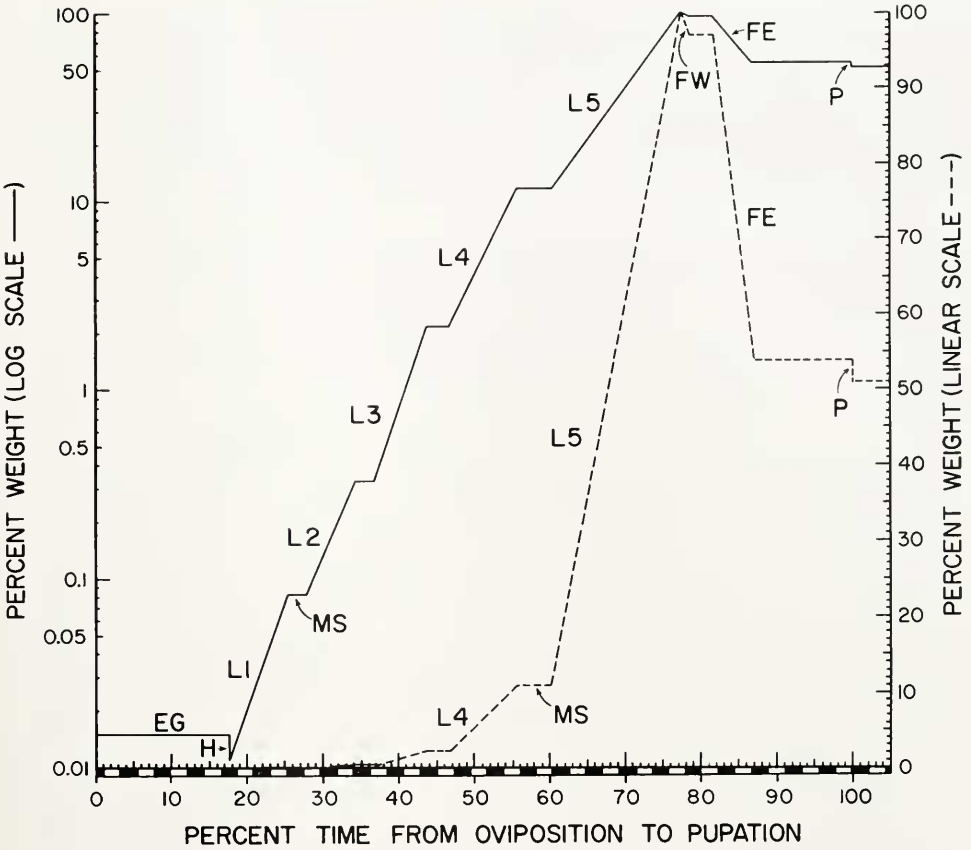

FIGURE 1. Rate of growth of physiologically advanced (PA) insects graphed on log (solid line) and linear (dotted line) scales; % weight scale (100% = 9.23 g) against % time (100% = pupation at 22.8 days). Dashes at base of graph represent 12-hr scotophases of each day at 25° C, starting at 6:00 PM. Numbers preceded by L identify instar. EG = egg stage; FE = fluid excretion ;FW = period of estimated weight loss due to end of frass elimination and use of fluid for body wetting; H = hatch; MS = molt sleep plateaus between end of feeding stage and larval-larval ecdysis; P = pupal ecdysis. All points were derived from percentage column of Table I and weight column of Table II and simply connected.

ecdysis, and then the larvae remained motionless for up to about five hours or until the onset of the scotophase.

Heart exposure. The dorsal aorta became visible approximately three hours after feeding stopped. The aorta became very obvious by the end of the next event—body wetting—and it remained so until pupal ecdysis. This visually observable event has been used frequently and is valuable as the first prodromal sign of pupation for larval *M. sexta* (Truman and Riddiford, 1974; Nijhout, 1976). However, its occurrence and detection can vary by more than 5 hours.

Body wetting. Within about 8 hours after feeding stopped, the larvae began "body wetting." This phenomenon (but not the term) was first reported by Baumhover, Cantelo, Hobgod, Knott, and Lam (1977). Time-lapse studies showed that this activity is characterized by a sharp turning of the head so that all accessible dorsal and lateral body surfaces can be covered with an oral secretion.

The insects initiated this wetting at the thorax and proceeded to cover the surface of the body to the end of the abdomen on one side and then repeated it on the other side, taking from 5 to 15 min to complete each side; the sides were alternately moistened three or four times. Mean duration of body wetting was 2.7 hr, and it tended to begin about 7:00 PM. Defecation continued throughout this stage and resulted in the production of about six fecal pellets after cessation of feeding.

Insects that were physiologically ready for body wetting started this activity at the onset of lights off. The remaining insects entered an increased state of activity until they, too, were capable of body wetting (as much as several hours later). This was the only instance we noted in which a change in lighting altered behavior. This effect was subsequently used to derive a new and better synchronized 0 time, which started with body wetting at 0 A.Z.T. A more precise synchronization of post feeding of *M. sexta* was obtained by simply selecting larvae 4 days after ecdysis and shortly before lights off and which showed the first signs of heart exposure but did not display a wet body. These insects were again observed 1 hr or so after the onset of the scotophase. Those showing body wetting characteristics (e.g., wet body or sharp turning) were selected as physiologically synchronized to within 1 hr of each other. Film records of six larvae showing the onset of body wetting within 15 min of lights off ended body wetting activtiy 3.25 ± 0.18 hr later, and all pupated 5.10 ± 0.02 days from the new time 0.

Wandering. Larvae began the wandering stage (Truman and Riddiford, 1974) immediately after body wetting activity ceased. Wandering activity was continuous, and the duration depended upon the condition of the container. When the cup was cleared of diet and frass soon after body wetting, wandering activity lasted about 32 hr. However, when residue was left in the container, duration of wandering was reduced to 8.5 hr (Table II). When the larvae were allowed to end body wetting on soil, wandering was occasionally eliminated and replaced by burrowing behavior.

Dorsal pigmentation. A reddish pigmentation spread slowly over the dorsal surface of the larvae at the end of body wetting or shortly after the onset of wandering for about 12 hr and then diminished at about the same rate. Coloration was most apparent a few hours after the onset of wandering (or burrowing). However, some larvae had no noticeable pigmentation.

Burrowing. As noted, when residue was left in the containers, the larvae changed their behavior from wandering to burrowing, the onset of which was about 11 hr after the start of body wetting; burrowing activity lasted for 23 hr. Toward the last half of this stage, the larvae formed a pupation chamber from the mixture of frass and food. When they were placed in the narrow soil chamber, film records showed that the larvae burrowed just under the surface for several hours before pursuing a zigzag course to a depth of 6 to 20 cm, after which they formed a horizontal earthen cocoon.

Fluid excretion. The fluid excretion phase (termed gut purge by Nijhout and Williams, 1974), like heart exposure and dorsal pigmentation, overlapped major events. The larvae excreted a clear, viscous fluid from the anus in several discrete discharges over a period of 28 hr. Such discharges started about 20 hr after the onset of body wetting and occurred every 5.4 ± 5.3 hr (n = three insects with a total of 20 discharges) and were responsible for the loss of nearly 40% of larval weight. Observations reported here were made while the insects were confined in containers lined with brown paper. Larvae burrowing in the soil used this

discharge to dampen the surrounding soil as they packed the mixture to form an earthen cocoon.

Reduced movement. Approximately 34 hr after the onset of body wetting, the larvae immediately entered a period of reduced locomotor activity that lasted for 12.9 hr. Larvae held in containers lined with damp paper frequently scraped the paper with their mandibles. This activity probably represented the last stage in the preparation of the earthen cocoon.

Stationary stage. Reduced locomotion was followed by a stage where the insects were unable to move forward or backward. This stage began about 47 hr after the onset of body wetting and was signaled by a characteristic movement of the head and tail regions in opposite directions so as to form a Z or S shape. Alterations in body positions, of which there were 58 ± 5 ($n = 9$ specimens) occurred every 1.5 to 2 hr through most of this stage but increased in frequency as the insects approached pupation. Duration of the stationary stage to pupation was 2.0 days (Table II).

Metathoracic bars. At 4.2 days after body wetting or 16 hr before pupation, two areas on the metathorax, one on each side of the heart perpendicular to the axis of the larvae, began to melanize. After 6 hr, these areas were prominently melanized marks.

Larval-pupal ecdysis. Larval-pupal ecdysis occurred about 5 days after the onset of body wetting (Table I). Three postpupation observations were made, starting with proboscis growth. Formation of the proboscis, which was attached at the tip of the mid-thorax, was aided by being stretched from the head as the insect straightened from a head down posture to full length. The proboscis was fully formed 1.8 ± 0.5 hr ($n = 9$) after ecdysis. Melanization from the proximal end was half completed 2.6 ± 0.5 hr ($n = 9$) after ecdysis and complete melanization of the proboscis occurred 4.1 ± 0.6 hr ($n = 8$) after ecdysis. Riddiford and Ajami (1973) published a detailed description of the characterization of *M. sexta* during the first 24 hr after larval-pupal ecdysis at 26° C. Their times of occurrence for formation, and half and complete melanization of the proboscis, were reported at 1.25, 1.50, and 3 to 3.50 hr after ecdysis, respectively.

Pupal-adult ecdysis. Diapause-entrained adults eclosed an average of 97 days after pupation. However, this varied and depended upon environmental conditions and genetic makeup of the colony. Nondiapause insects eclosed about 22 days after larval-pupal ecdysis (Table II).

A time-lapse study of an insect eclosing within an earthern cocoon 20 cm below the surface of the soil showed an adult that freed itself from the pupal skin in less than 30 sec and immediately discharged about one ml of meconium. The relase of this liquid waste softened the earthen cocoon and facilitated escape. Young moths were capable of producing another meconium discharge at any time up to the first flight. The adult made a vertical ascent to the surface in about 10 min under existing conditions.

Film records of unrestricted eclosion in a 15-cm transparent cube with a diagonal 25 cm stick showed newly emerged moths had found a wing-spreading site on the stick 11 ± 7 min ($n = 8$) after eclosion. The moths immediately began a dorso-lateral revolution of the wings and showed the first slight signs of wing inflation. The rotation ended in about 2 min, with the wings held in a "butterfly" position. This was evenutally followed by accelerated wing inflation activity, which was completed 33 ± 11 min ($n = 8$) after eclosion. At 1.2 ± 0.1 hr ($n = 7$) after eclosion, the moths brought the wings to the characteristic "tent"

position. Adults tended to use their wings 3.5 ± 0.2 hr ($n = 6$) after eclosion. The second discharge of meconium was usually released moments before or at this time. Timed events reported here with standard deviations corresponded well to similar events reported by Truman and Endo (1974).

Discussion

There are a number of factors that can affect the growth and development rate of an insect colony. Such factors include temperature, humidity, photophase, basic diet, diet preparation, source of dietary ingredients, antimicrobial agents, genetic strains, size of insect containers, competition for living or feeding space, and noise. Other factors presently too subtle to understand also appear to affect growth rate of laboratory-reared insects. For example, Nijhout and Williams (1974) attributed some variability in the *M. sexta* colony to the time of year. To help obtain a more precise study of the growth and development of a *M. sexta* colony, we employed methods that reduced commonly encountered variables and misinterpretations. Such methods included the use of stable nutrition, time-lapse cinematography, filmed recordings of nutiple insects, close monitoring of time and temperature, and the selection of a specific group of insects within the colony—the most physiologically advanced group.

Data from the percentage column of Table I may be used to derive a new growth rate at a temperature other than 25° C. This can be accomplished by carefully determining onset time of one or two events such as pupation or body wetting for PA insects reared under a different constant temperature. Such preliminary temperature studies augmented by time-lapse films have allowed the development of the equation: $Y = N[0.0061 + 0.0980\ (25 - T) + 0.0085\ (25 - T)^2]$, where N represents any time of onset in Table I, and T is the new temperature in centigrade. This formula can be used to estimate the onset times of events in insects reared at different temperatures. (Gauss-Newton method used to derive the three parameters of the equation from seven events observed at 22°, 25°, and 28° C).

Thus, the results of this study should be directly applicable in most instances to other colonies of *M. sexta* to increase the awareness of behavior and the synchrony of the life cycle for better use in research.

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Summary

1. Time-lapse cinematography was used with laboratory-reared specimens of *M. sexta* to obtain information on and timing of behavioral activities.

2. Insects used in this study were selected as the most advanced group within any instar and thus were the first of the gate-I insects. Use of these insects avoided timing irregularties involving the gating phenomenon. These advance insects varied less than ±0.9% s.d. from hatch through pupation.

3. A detailed description was given for each of more than 20 timed events. Emphasis was placed on the events that occurred between the fifth ecdysis and pupation.

4. Hatch occurred four days after oviposition (time 0), and ecdyses of the remaining four larval instars were at 6.4, 8.4, 10.6, and 13.7 days; pupation occurred at 22.8 days. The larvae underwent molt sleep in which they did not feed, defecate, or move until ecdysis. Durations of molt sleep were from 14 to 25 hr for each of the larval-larval ecdyses. The fifth instar did not have a comparable stage.

5. Growth rate was highest for the first-instar larvae and slowest for the fifth instar, with weight-doubling times of 11.3, 17.6, 11.4, 17.3, and 21.2 hr for the first through fifth instar feeding periods, respectively, at 25° C.

6. Body wetting, found only in the last instar, was induced by the onset of the scotophase. Use of this event marker allowed the selection of a group of insects that were capable of pupating five days later within one hour of each other.

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